RESPONSE

This response addresses the issues raised in the last office action in the pending application. The sole remaining rejection is a § 103 rejection based on the combination of the Pain, Clark, Shen, Nakazawa, Sakurai, Samarut and Etches (1996) paper in Development (Pain, Etches et al.), the Petitte et al United States Patent (USP 5,830,510), the Gibbins et al. (1990) reference (Gibbins et al.).

The Examiner has asserted that the claimed chimeric birds are indistinguishable from those recited in the art. This allegation of fact, and many of the other factual assertions upon which the § 103 rejection is based, is demonstrably untrue. The prior art does not disclose a single instance of a chimeric bird harboring a "stably integrated transgene" having detectable expression in the tissue type of the animal. Moreover, the enabling technologies consisting of transformed cells and a demonstration of long term cultures of such cells is not disclosed in the art. The claimed invention is a totally novel invention and is not disclosed, enabled, or taught by the cited references, either alone or in combination as of the invention date of the present claims. On this point, the Office Action seems to treat the existence of transformation attempts for embryonic stem cells, and the ability to create chimeras from cultured cells demonstrating any glimpse of the donor phenotype as the claimed invention. Clearly, the claimed invention distinguishes the most advanced development of chimeric birds disclosed in the cited prior art references. The prior art simply does not disclose any embryonic stem cell culture bearing a stably integrated transgene maintained in culture for any usable length and does not disclose any cells in culture bearing a transgene that contribute to the genome of a resulting chimeric bird.

Accordingly, the rejection must be withdrawn because: 1) the Examiner's reliance on the factual content of particularly the Pain, Etches et al. and Gibbins et al. references is demonstrably in error; 2) these errors cause a fundamentally erroneous conclusion on the patentability issue -- both the factual

content of the *prima facie* case and the ultimate legal basis for the § 103 rejection; and 3) the Examiner does not appear to have considered the secondary considerations evidence in the record.

Finally, contrary to the statement in the Office Action, the pending claims are plainly not product-by-process claims. Claims 2, 26-30 are product claims that do not rely on process limitations. Claims 41-50 are traditional method claims.

Because the Office Action fails to distinguish between the method claims of the present application and the product claims of the present application when reciting the alleged *prima facie* for obviousness, Applicants cannot discern when the Examiner is making arguments that are particular to the method claims, versus those particular to the product claims. However, the product claims and the method claims both require the successful creation of a chimeric chicken expressing a stably integrated transgene – a construct and a method totally unknown in the prior art prior to the present invention.

I. THE REJECTION IS BASED ON ERRONEOUS FACTUAL ASSERTIONS REGARDING THE PAIN, ETCHES ET AL. REFERENCE.

The Examiner bases the § 103 rejection on a number of statements of fact that are demonstrably in error. For example, the Examiner states:

> Pain [(Etches et al 1996)] teaches in vivo differentiation of chicken embryonic stem cells (CEC) obtained from long term culture.

(Office Action at p. 3).

This statement is incorrect because the ability of Pain, Etches et al. to make germline chimeras was restricted to 7 days and the ability to make somatic chimeras was restricted to 21 days. To be considered "long term," cultures must be actively growing and expanding for at least one month. See Etches Affidavit ¶ 5-6 04/28/2004 (attached hereto as Exhibit A). The sworn declaration of Dr.

Etches, a named inventor of the present application and an author of the prior art reference relied upon by the Examiner, directly rebuts the statement of fact that the Examiner attributes to the content of the reference. If the Examiner has an evidentiary basis for asserting a fact that is contrary to a sworn declaration by the author of the reference, it has not been provided.

Furthermore, Pain, Etches et al. did not show in vivo differentiation other than the ability of the cells to contribute to feather pigmentation. Pain, Etches et al (1996) state that "The cells can differentiate into at least ectodermally, mesodermally and endodermally derived tissues *in vitro* and at least ectodermally derived tissues *in vivo*." (Page 2346 col 2 last paragraph). In contrast, the present invention claims differentiation into ectoderm, mesoderm, extra-embryonic and endoderm tissue *in vivo*. (See dependent claims 26-30, 45-50).

Furthermore, the Examiner states:

> The cited art further teaches that regardless of the number of passages more than 50% of the hatched recipient embryos were chimeras with nearly 33% of the plumage from donor phenotype (page 2344 col. 2 para 2; page 2346 Fig 8).

This statement is misquoted and taken out of context. To be taken in context, the actual and accurate quote should be read and considered verbatim and in its entirety:

> "Cells were collected from cultures after 1-3 passages. 100-500 cells were injected into the subgerminal cavity of irradiated stage X White Leghorn recipient embryos. Several of the grafted embryos hatched, some of which exhibited a chimeric plumage phenotype (Fig 8). Regardless of the number of passages, more than 50% of the hatched recipient embryos were chimeras with nearly 33% of the plumage color from donor phenotype."

Hence, the clear meaning of the sentence is that chimeras could be produced within the first three passages. The final statement of the paragraph explicitly states:

"The ability of long-term cultures to give rise to chimeric animals is currently under investigation".

Moreover, the legend to Fig 8. reads

"Chimeric chickens generated after grafting of cultured CEC (A) control normal White Leghorn chick (B-D) chimeric chicks derived from White Leghorn embryos grafted with Barred Rock CEC cultivated 3 to 19 days".

Clearly, chimeras could not be made from cells that had been in culture for longer than 19 days as is explicitly stated in the caption to Figure 8 of the reference. See also Etches Affidavit ¶¶ 5-6, 9. The facts recited in the Etches Affidavit are unrebutted in the present record and completely eliminate the factual bases underlying the pending § 103 rejection based on Pain, Etches et al. The Examiner cannot possibly cite evidence from this reference that chicken ES cells could be cultivated for periods sufficiently long to establish a stably transfected line of ES cells. Because the reference at page 2346 explicitly states: "Work is in progress to improve the culture conditions allowing clonal selection of stem cells and further work is required to improve donor-derived cells so they can enter readily and at a high frequency into the germline." These data are also corroborated by Petitte et al, *Mechanisms of Development* 121: 1159-68 (2004) (Attached as Exhibit B) which showed that germline transmission could not be maintained for ES cells held more than 7 days in culture and that only minor somatic contributions (judged by feather pigmentation) could be obtained after 21 days in culture.

The cited Pain, Etches et al. prior art does not contain any example chimeras from ES cells stably transfected with a transgene. The phrase in Pain, Etches et al. "regardless of the number of passages," clearly refers to "Cells (that) were collected from cultures after 1-3 passages". Beyond this time, the cells of Pain, Etches et al. lost their ability to contribute to somatic tissues. This is evident from the last sentence of the Pain, Etches et al. paper which states: "The conditions that support

proliferation and differentiation of chicken blastodermal cells have been established opening the way to isolation and utilisation of avian pluripotential embryonic stem cells."

Therefore, it is absolutely beyond dispute that the underlying factual basis for the sole rejection of this application cannot stand because Pain, Etches et al. did not have the ability to make chimeras with genetically modified cells. These authors explicitly state "We have been unable so far to maintain clonal growth of CEC...." See p. 2346 (penultimate sentence). Thus, if Pain, Etches et al. were unable to maintain clonal growth, it is beyond dispute that Pain, Etches et al. were unable to derive transfected cell lines and therefore make chimeras with transfected ES cells. Since the present claims recite "...chimeric chickens comprised of progeny of embryonic stem cells having a genome comprising a stably integrated transgene...," Pain, Etches et al. cannot disclose the long term cells capable of harboring a stably integrated transgene. Again, the Etches Affidavit at ¶ 5-9 is unequivocal on this point. As noted previously, the production of genetically modified chicken ES cells would not be obvious to one skilled in the art because such cells were not invented until the disclosure of the present application and for another 7 years after the Pain, Etches et al. publication despite being sought after by every group working in the field (see Petitte, J. N., Liu, G. Yang, Z., (2004), Avian pluripotent stem cells, Mech Dev. 121, 1159-1168; Sang, H. (2004), Prospects for transgenesis in the chick, Mech. Dev. 121, 1179-1186, Ivarie, R., (2003), Avian transgenesis: progress towards the future, Trends Biotechnol. 21, 14-19, Zajchowski, L. and Etches, R.J., (2000), Transgenic chickens: past present and future, Poultry and Avian Biology Reviews, 11, 63-80).

Still further, the Examiner states:

> The cited art further teaches CEC expressing ECMA-7, SSEA-1 and EMA-1 could be cultured for at least 35 passages i.e. more than 160 days in the presence of LIF...."

This statement describes *in vitro* attributes of the cells. The present claims recite a "hatched chicken" and require the ability of genetically modified cells to contribute to embryos *in vivo*. As described above, Pain, Etches et al. (1996) were explicitly and admittedly unable to make hatched chimeric animals with cells that were in culture for more than 19 days. The above statement says nothing about expression of a transgene contained in the CEC in a resulting chimeric bird and has no relevance to the pending claims.

Still further, the Examiner states:

> ...The cited art teaches that it is well known in the art that cells derived from the chicken blastoderm will contribute to both somatic tissues and the germline when injected into recipient embryos to form chimeras (page 2339 col 2 par 2).

These references merely provide data showing that cells taken from the stage X blastoderm contribute to both somatic tissues and the germline. However, they do not teach that cultured cells from the stage X blastoderm contribute to embryonic tissues *in vitro* nor do they teach methods to genetically modify cells.

To summarize, the Pain, Etches et al. (1996) paper teaches a culture containing cells which could produce chimeras, within 3 passages, when maintained in culture for 19 days or less. For a short period of time (i.e. up to 19 days), these cells could make somatic chimeras, and for 7 days, these cells could make germline chimeras. However, after longer periods in culture, they could make neither somatic nor germline chimeras. All of these attributes are described in the paper and stand in sharp contrast to the interpretation by the Examiner and to the content of the present claims.

II. THE REJECTION IS BASED ON ERRONEOUS FACTUAL ASSERTIONS REGARDING THE PETITTE ET AL. REFERENCE.

Now, turning to the Petitte reference, the Examiner states:

> Petitte (5,830,510)...further teaches genetic modification of avian embryonic stem cells by transfecting avian embryonic stem cells with the DNA sequence in vitro ....and then injecting the transfected embryonic stem cells into an egg containing an embryonic bird (col 4 lines 7 – 30).

The citation to Petitte on this point is clearly not evidence to support a *prima facie* basis under § 103, but rather conspicuously shows a failed attempt by others to invent the subject matter of the present claims. Clonal derivation of genetically modified ES cells was never shown in this patent, in any of the family of patents by this inventor, or in any scientific publication. Referring again to Exhibit B, as late as 2004, this author/patentee showed results demonstrating chimeras up to only 20 days, which, of course, could not contain a stably integrated transgene, nor contribute to a chimer bird containing a transgene as claimed. The first invention of genetic modification of avian embryonic stem cells and stable integration into a chimera is the instant application.

Further, the Examiner states:

> The cited art further teaches culturing and maintenance of avian embryonic stem cells for 23 passages which is approximately 2 months (60 days) see col 8, lines 5-9, table 1.

Again, and clearly, this text merely describes attributes of the cells growing *in vitro*. It is abundantly clear and beyond dispute that the behavior of non-transformed embryonic cells *in vitro* may does not predict their behavior in a recipient embryo and mere growth in culture does not indicate the potential of the cells to receive and integrate a stably integrated transgene, to continue to grow and be selected in culture following integration to contribute to the genome of a recipient embryo, and does not indicate the potential to produce the successful hatch of a chimeric bird expressing the transgene. The perfect example of this obvious fact is the Pain, Etches et al. work discussed above where cells grown in culture lost the ability to contribute to chimeras at 19 days.

III. THE REJECTION IS BASED ON ERRONEOUS FACTUAL ASSERTIONS REGARDING THE GIBBINS ET AL. REFERENCE.

Regarding the Gibbins et al. reference, the Examiner states:

> Similarly, Gibbins et al teaches efficient transfer of chicken blastodermal cells and their incorporation into recipient embryos to produce chimeric chickens.

Gibbins et al. merely teach transfer of blastodermal cells, but not embryonic stem cells. Blastodermal cells are very different cell types with different morphologies and very different properties. Hence, the Gibbins et al. prior art reference is not relevant to a *prima facie* case under § 103 to the pending claims.

Furthermore, the Examiner states:

> The cited art teaches isolation and culture of pluripotent embryonic stem cells and transfection of these cells with DNA constructs using Lipofectin.

Gibbins et al. do not teach any viable technology that could be practiced with embryonic stem cells because Gibbins et al. did not use embryonic stem cell lines. The main thrust of Gibbins et al. is the use of blastodermal cells as surrogates for embryonic stem cells, and the conspicuous failure to derive pluripotent embryonic stem cells from blastodermal cells. Again, the behavior of one type of cells in culture, particularly cells of dissimilar type and morphology, does not predict the behavior of another cell type in transgenesis and one or ordinary skill in the art would not have any expectation of success applying the pertinent technology from blastodermal cells to embryonic stem cells.

Still further, the Examiner states:

> The cited art further teaches selection of stably integrated transfected cells and introduction of the transfected cells into recipient embryos to generate chimeric birds (page 120 para 2-3).

This is demonstrably false. Gibbins et al. have no transfected cells and cannot introduce transfected cells into recipient embryos because transfected cells did not exist prior to this invention. On the contrary, Gibbins et al (1990) expressly state:

> "The efficient production of specific lines of transgenic birds via chimeric intermediates requires the development of several processes:
>
> 1. isolation and culture of pluripotent embryo cells or primordial germ cells,
> 2. efficient transfection of these cells with DNA constructs,
> 3. development of constructs capable of undergoing homologous recombination with the genome,
> 4. selection of stably transfected cells,
> 5. introduction of these transfected cells into a recipient embryo to generate a chimeric bird,
> 6. rapid identification of germline chimeras, and
> 7. incorporation of transgenic birds into commercial breeding programmes.
>
> "In partial fulfillment of these objectives, we have produced somatic and germline chimeras following transfer of chicken stage X blastodermal cells into stage 10 recipients. [citation omitted]"

Clearly, the authors did not have lines of embryonic stem cells, did not have the ability to stably transfect them, did not have the ability to select stably transfected cells, and did not have the ability make chimeras with transfected cells. Therefore, Gibbins et al. (1990) does not disclose selection of stably transfected cells, nor the production of chimeras from these cells. Gibbins et al. (1990) specifically discloses that the goal has yet to be achieved. Gibbins et al. (1990) state:

> "A major problem that we have encountered is that we have not been successful in culturing chicken embryonic stem cells for any extended period of time without differentiation taking place."

Again, prior to the present invention, the genetically modified embryonic stem cells necessary to make the chimeric birds of the invention did not exist and the Examiner's proposition that any of the

teachings of Pain et al., Gibbins et al., or Petitte et al. establishes that ES cells could be genetically modified cannot stand because none of the references disclose genetically modified cells. Directly to the contrary, each and every reference relied upon by the Examiner shows that an ES cell culture could not be maintained "for any extended period of time" such that transfection and selection could take place while the cells maintained the ability to contribute to a chimeric animal.

Still further, the Examiner states:

> The cited art further teaches successful construction of chimeric birds from several different donor/recipient line combinations indicating that the technique should have broad applicability across breeds (page 121, para 2).

These data are not relevant to the present claims because they have nothing to do with long term cultures of ES cells having stably integrated transgenes, and have nothing to do with ES cell derived transgenes displaying expression in a chimeric chicken. Gibbins et al. were removing blastodermal cells (not embryonic stem cells) from stage X embryos and making the chimeras before the cells were cultured. Several breed combinations were tested, but no equivalence exists between blastodermal cells from different breeds and lines of embryonic stem cells from different sources or lines of embryonic stem cells that have been clonally derived with transgenes stably integrated into the genome. It is beyond the scope of one skilled in the art to be able to extrapolate from breeds of chickens to cell lines.

Finally, the Examiner states:

> One would have a reasonable expectation of success since genetic modification, long term culture of embryonic stem cells and making chimeric birds using embryonic stem cells has been routine in the art.

This statement ignores the limitation of the claims that a transgene is stably integrated and expressed. The entire history of the field as disclosed in the references of record, as well as common

knowledge in related transgenics applications in mice, pigs, cows, rabbits, etc. clearly shows that significant technical hurdles exist to: (1) establish lines of embryonic stem cells from chicken blastodermal cells; (2) make uniformly genetically modified lines of stably integrated embryonic stem cells as opposed to unselected lines of transiently transfected blastodermal cells; (3) use lines of stably transfected embryonic stem cells to make chimeras as opposed to using transiently transfected blastodermal cells from different breeds of chicken; and (4) achieve expression of the transgene in somatic tissue types. The development of stable genetic modifications in chicken embryonic stem cells and the production of chimeras that express the stable modification could not have been expected based on failed attempts to culture long term ES cells (Petitte et al.), discovering time limits for the length of ES cell cultures contributing to chimeras (Pain, Etches et al.), and failed efforts to create chimeras using dissimilar cell types (Gibbins et al.).

Also, as noted above, the Examiner also cannot maintain that the claimed chimeric chicken is indistinguishable from or would have been obvious from the prior art. The clearest demonstration of the novelty and non-obviousness of the present invention is that prior art cell cultures do not exist in culture long enough for transformation and prior art chimeras do not have a stably integrated transgene. The Examiner has not, and cannot, cite an example in the prior art of a chimera having a transgene because none existed prior to the present invention.

Therefore, in conclusion, the Examiner's rejection of the pending claims cannot stand because the rejection is based on a fundamental misinterpretation of the content of the Pain, Etches et al. paper, the Petitte et al. patent, and the Gibbins et al. reference and is contrary to the facts established by the Etches Affidavit and the remainder of the record of this application.

IV. THE PRESENT INVENTION SATISFIES A LONG-FELT NEED IN THE ART AND OVERCOMES PRIOR FAILED ATTEMPTS BY OTHERS.

Although the Applicants believe that the evidence of record establishes that secondary considerations evidence is unnecessary because the Examiner has not established a *prima facie* case for obviousness under § 103 that would shift the burden of proof to the applicant, and although Applicant believes that the secondary considerations evidence is so conspicuous in the record that it should have been considered in the Examiner's rejection, Applicants are setting forth here a separate discussion of the secondary considerations evidence to be sure that the record is complete and that the evidence is properly considered in the context of the § 103 analysis.

Even if one considers only the time period from the Gibbins et al. reference (1990) until the filing date of the present application, a period of 12 years exists wherein skilled researchers expressed the desire but were unable to prepare and maintain a long-term cell culture that was capable of successful integration of a transgene. Moreover, the limitations on the ability of embryonic stem cells existing in a cell culture to contribute to the germline of a recipient embryo prevented achieving the present invention. As noted, this limitation was based on the limited time period in which chimeras could be created and was insufficient for any transgenic manipulations of the target embryonic cells. See Etches Affidavit ¶ 6-7.

Furthermore, it is readily apparent from the content of the references, and particularly from the patent portfolios of Petitte et al. (see, e.g., USP 5,830,510) that the principal goal of this work was to achieve the results now described and claimed in the present application. However, as recently as 2004, these same researchers in the field of avian pluripotent stem cells described attempts to make chimeras from embryonic stem cells in culture and did not report the creation of chimeras from any culture

longer than 20 days. Petitte et al. *Mechanisms of Development 121:* 1159-68 (2004), see Table 1 (attached as Exhibit B). As described below, this fact establishes the requisite nexus between the secondary consideration's evidence and the non-obviousness of the claimed invention.

The mandate of the Federal Circuit case law requires that secondary consideration's evidence of nonobviousness must be considered when ruling on the issue of obviousness. *Simmons Fastener Corp. v. Illinois Tool Works*, 739 F.2d 1573, 1575 (Fed. Cir. 1984). The Federal Circuit recognizes that secondary considerations of nonobviousness, "may often be the most probative and cogent evidence in the record." *Id.* Additionally, evidence of secondary considerations of nonobviousness may be used to rebut a prima facie case of obviousness based on prior art references. *WMS Gaming, Inc. v. International Game Technology*, 184 F.3d 1339, 1359 (Fed. Cir. 1999). The present record contains evidence both of a long-felt, but unmet need, and prior failed attempts by others. Each body of evidence overcomes any conclusion that the claims at issue were obvious. See Etches Affidavit, ¶ 5-9. Also, the repeated "failure of others," which is properly viewed as a "failure of others to achieve the claimed invention," readily demonstrates that the presently claimed invention would not have been obvious. See Advanced Display Systems v. Kent State University, 212 F.3d 1272, 1285 (Fed. Cir. 2000) ("repeated failures to design the claimed invention" was persuasive evidence of nonobviousness). See also Transmatic, Inc. v. Gulton Indus., Inc. 53 F.3d 1270, 1275 (Fed. Cir. 1995) (noting that "the failure of others to achieve the patented invention" supported conclusion of nonobviousness); Symbol Tech., Inc. v. Opticon, Inc., 935 F.2d 1569, 1578 (Fed. Cir. 1991) (evidence of "the failure of others to develop the claimed invention" is an objective indicia of nonobviousness); Dow Chemical Co. v. American Cyanamid Co., 816 F.2d 617, 622 (Fed. Cir. 1987) (competitor "failed to develop the claimed invention"). The requisite nexus between the secondary consideration's evidence and the claimed invention is the fact, described

repeatedly in the present record, that the present Applicants were the first to enable embryonic stem cell cultures, having stably integrated transgenes, that contribute to the genome of chimeras and wherein the transgene is broadly expressed in somatic tissue.

Applicants contend that the pending claims are in condition for allowance and request such action accordingly.

The Commissioner is authorized to charge to Orrick Herrington & Sutcliffe's Deposit Account No. **150665** the amount of **$510.00** for the three-month extension fee. The Commissioner is authorized to charge any additional fees required by the filing of these papers, and to credit any overpayment to Orrick Herrington & Sutcliffe's Deposit Account No. **150665.**

Respectfully submitted,

ORRICK, HERRINGTON & SUTCLIFFE LLP

Dated: November 23, 2005 By: _____

Kurt T. Mulville
Reg. No. 37,194

4 Park Plaza, Suite 1600
Irvine, CA 92614
949/567-6700 Telephone
949/567-6710 Facsimile